COMPLEMENT FIXATION IN HOG CHOLERA*

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During the summer of 1914, while working on the etiology of hog-cholera, we found it desirable to try the complement-fixation test, using as antigen a certain micro-organism which had been isolated. These tests were not successful. This organism had been isolated from the mesenteric glands of acute cholera hogs. The pathologic changes in such glands were frequently so marked that it seemed highly probable that the cholera virus was present in them to a marked degree. Therefore, one of us (Healy) attempted the preparation of an antigen by extraction of these glands in the following manner:

The mesenteric glands, which were enlarged, dark in color, and congested, were obtained from three acute cholera hogs which had been bled for virus. The glands were dissected from the surrounding tissues and ground with sterile sand. The resultant mass was then placed on ice over night at a temperature of 4 C. On the following day, a 1 percent neutral glucose beef broth was added to the ground glands in the proportion of one part by weight of unground glands to ten parts by weight of glucose broth, and the mixture again placed on ice at 4 C. for eight days. At the end of this period, a portion of the mixture was passed through a Royal Berlin porcelain, open-topped, Pasteur-Chamberland filter—bougie E. & A., 1802. The filtrate, of a pale amber color and perfectly clear, was used, unheated, as antigen. All manipulations were performed as aseptically as possible. The hog serum used was an amber-colored serum obtained from a hyperimmune hog. This serum had been on ice for four months. The first complement-fixation test resulted as follows:

Antigen in c.c.	Normal Salt Solution in c.c.	Immune Serum in c.c.	Complement in c.c.	Hemolysin in c.c.	Red Blood Corpuscles in c.c.	Hemolysis
0.10	1.5	0.05	0.045	0.15	0.5	Complete
0.13	1.5	0.05	0.045	0.15	0.5	None
0.15	1.5	0.05	0.045	0.15	0.5	None
0.17	1.5	0.05	0.045	0.15	0.5	None
0.20	1.5	0.05	0.045	0.15	0.5	None
0.25	1.5	0.05	0.045	0.15	0.5	None
0.30	1.5	0.05	0.045	0.15	0.5	None

The antigen, immune serum, and complement were mixed and placed at 37 C. for one hour, where pon the hemolysin and corpuscles were added and the whole incubated at 37 C. for two hours.

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The test for complement absorption was negative. The tests for antigen, serum, complement, and lysin resulted as follows:

Antigen in c.c.	Normal Salt Solution in c.c.	Immune Serum in c.c.	Complement in c.c.	Hemolysin in c.c.	Red Blood Corpuscles in c.c.	Results
0	1.5	0.05	0.045	0.15	0.5	Complete
0.65	1.5	0	0.045	0.15	0.5	Complete
0.65	1.5	0.05	0	0.15	0.5	None
0.65	1.5	0.05	0.045	0	0.5	None

The test was repeated on the following day with the same results.

After this antigen had stood on ice at a temperature of 4 C. for twenty-three days, it was again tested with the following result, the test being made in exactly the same way as before:

Antigen in c.c.	Hemolysis
0.10	Complete
0.13	Complete
0.15	Nearly complete
0.17	Partial
0.20	Trace
0.25	None
0.30	None

The antigen, now passed through an imported Pasteur-Chamberland "F" bougie, lost the power to fix complement in the strength shown in the preceding table.

It is of interest that the strength of the antigen diminished with time, and, indeed, when tested on the twenty-sixth day, gave a negative reaction throughout. This alteration of antigen was possibly due to slight micro-organic growth. It is of considerable interest also that the imported "F" bougie removed the antigen altogether.

There can be no doubt that a reliable complement-fixation test for hog-cholera would be of value, not only as an aid in the study of this disease, but also as a means of diagnosis in those cases of chronic hog-cholera in which malnutrition is the only symptom. Possibly also such a test might be used successfully in the standardization of hyperimmune serum and the demonstration of immunity in a given hog.

On further study of this test, we were impressed with the following facts: That the mesenteric glands used must show distinct pathologic changes, indicated by increase in size, darkness of color, and congestion; that the suspension of such ground glands in the 1 percent glucose beef broth must remain at a temperature of 4 C. for at least eight days before the antigen develops; and that all manipulations must be as aseptic as possible.

Washed sheep blood corpuscles were used in the tests. The blood was drawn from the jugular vein through a large hypodermic needle and allowed to flow into a sterile bottle containing glass beads. The blood was immediately and thoroughly shaken for five to ten minutes to defibrinate it. It was then filtered through sterile cotton into 100 c.c. centrifuge tubes, about 20-30 c.c. of blood being placed in each tube. The tubes were next filled with sterile, normal salt solution and centrifugated at 4,000 revolutions for twelve minutes, the supernatant liquor pipetted off, and the operation repeated. This washing was repeated four times, after which a 2 percent suspension of the corpuscles in normal salt solution was prepared. We used 0.5 c.c. of such a suspension in the tests described.

The clear serum of a rabbit which had received repeated injections of sheep corpuscles, washed as above, was used for hemolysin. Ten cubic centimeters of washed sheep corpuscles were mixed with an equal quantity of sterile, normal salt solution, and injected intraperitoneally into the rabbit at eight-day intervals. The rabbit received six such injections. Eight days after the last injection, a small quantity of blood was withdrawn from an ear vein, and after the clotting of the blood the serum was tested. If the serum was of sufficient strength, the rabbit was bled and the clear serum obtained from the clot. This serum was heated to 56 C. for thirty minutes to destroy complement, and then was preserved by the addition of 0.2 percent carbolic acid, and kept on ice. In our tests, 1 c.c. of the inactivated rabbit serum, added to 25 c.c. sterile, normal salt solution, was used as hemolysin. This mixture was titrated against 0.5 c.c. of the 2 percent washed blood corpuscle suspension and a quantity of complement of known strength. Thus:

Hemolysin in c.c.	Normal Salt Solution in c.c.	Complement in c.c.	Red Blood Corpuscles c.c.	Hemolysis
0.01	1.5	0.03	0.05	None
0.02	1.5	0.03	0.05	Partial
0.03	1.5	0.03	0.05	Complete
0.04	1.5	0.03	0.05	Complete
0.05	1.5	0.03	0.05	Complete
0.06	1.5	0.03	0.05	Complete

The titer of this hemolysin was 0.03, and the dose (five times the titer) was 0.15 c.c. This hemolysin was used in the first tests. The second hemolysin gave a titer of 0.02, the dose being 0.10 c.c.

Normal guinea-pig serum furnishes the strongest and most stable complement. In this experiment, the guinea-pig was bled from the throat and the blood allowed to flow through a sterile funnel into a sterile 50 c.c. centrifuge tube, where it rapidly clotted. The clot was separated from the side of the tube with a sterile glass rod and was then placed at 37 C. for thirty minutes to one hour. The separated serum was placed in small tubes and centrifugated until clear. The serum was then mixed with twice its volume of sterile, normal salt solution and titrated against a known quantity of washed sheep corpuscles, and a quantity of inactivated, sensitized rabbit serum of known strength. Thus:

Complement in c.c.	Normal Salt Solution in c.c.	Hemolysin in c.c.	Red Blood Corpuscles in c.c.	Hemolysis
0.01	1.5	0.15	0.5	None
0.02	1.5	0.15	0.5	Nearly complete
0.03	1.5	0.15	0.5	Complete
0.04	1.5	0.15	0.5	Complete
0.05	1.5	0.15	0.5	Complete
0.06	1.5	0.15	0.5	Complete

The titer of this complement was 0.03, and the dose (1.5 times the titer) was 0.045 c.c. The complement, if kept on ice at about 6 C., retained its strength for several days. The complement had to be titrated very day, however, as it generally lost its strength, and that sometimes rapidly.

Eighteen grams of selected mesenteric glands from acute cholera hogs bled for virus, were thoroughly ground with sterile sand; 180 gm. of neutral 1 percent glucose beef broth were added and the whole placed at 4 C. At the end of forty hours, a portion of this suspension was passed through an ordinary white filter paper, and tested, unheated, with wholly negative result. The hog serum was amber serum obtained from a hyperimmune hog. This serum had been on ice for ten months.

At the end of five days, another portion of this suspension was passed through an ordinary white filter paper and tested, again with negative result. The test for complement absorption was negative.

At the end of eight days another portion of this suspension of mesenteric glands was passed through an ordinary white filter paper. The filtrate was quite clear and of an amber color. This antigen was tested, unheated, with three different immune sera in the same way as that described. Serum 1 was from a hyperimmune hog, and had been on ice for ten months; Serum 2 was from another hyperimmune hog, and had been on ice eighteen days; Serum 3 was from a hog on a farm where all hogs were immune. The result was as follows:

Antigen in c.c.	Hemolysis with Serum 1	Hemolysis with Serum 2	Hemolysis with Serum 3
0.10	None	Complete	Complete
0.13	None	None	None
0.15	None	Trace	None
0.17	None	Trace	Trace
0.20	Trace	Trace	Trace
0.25	Trace	Complete	Trace
0.30	Complete	Complete	Complete
0.0	Complete	Complete	Complete

The test for complement absorption was negative.

To obtain absolutely normal hog serum is, at the present time, most difficult. While it is true that there are some farms on which hog-cholera as yet has not appeared, there is a strong probability that the hogs on these farms possess a certain degree of immunity, either inherited or acquired. After careful search, we selected three hogs which appeared as normal as one could reasonably expect to find. Clear, amber serum was obtained from each of these hogs

and tested according to the scheme outlined with the following results. The immune Serum I was used as a check, as were also normal rabbit serum and normal cow serum.

Antigen in c.c.	Hemolysis with Normal Hog Serum 1	Hemolysis with Normal Hog Serum 2	Hemolysis with Normal Hog Serum 3	Hemolysis with Immune Serum 1	Hemolysis with Normal Cow Serum	Hemolysis with Normal Rabbit Serum
0.10	Complete	Complete	Complete	Nearly complete	Complete	Complete
0.13	Nearly Complete	Complete	Complete	Nearly complete	Complete	Trace
0.15	Partial	Complete	Nearly complete	None	Complete	None
0.17	Trace	Partial	Trace	None	Complete	Complete
0.20	None	Partial	None	None	Complete	None
0.25	None	None	None	None	Complete	None
0.30	None	None	None	None	Complete	None
0.00	Complete	Complete	Complete	Complete	Complete	Complete

In this series the normal rabbit serum was a disturbing element. This rabbit serum, however, reacted in an irregular manner throughout; in the following tests, in which the quantity of antigen was uniform (0.1 c.c.) and the quantity of serum varied, the rabbit serum yielded more consistent results:

Quantity of Serum in c.c.	Hemolysis with Normal Hog Serum 1	Hemolysis with Normal Hog Serum 2	Hemolysis with Normal Hog Serum 3	Hemolysis with Normal Rabbit Serum	Hemolysis with Immune Serum 1
0.005	Trace	None	Partial	Nearly complete	Partial
0.008	Complete	Partial	Complete	Partial	None
0.01	Complete	Complete	Complete	Trace	None
0.02	Complete	Complete	Complete	Complete	None
0.03	Complete	Complete	Complete	Complete	Nearly complete
0.04	Complete	Complete	Complete	Nearly complete	Complete
0.05	Complete	Complete	Complete	Nearly complete	Nearly complete
0.10	Complete	Complete	Complete	Complete	Complete
0.20	Complete	Complete	Complete	Complete	Complete

A thorough search of the literature has failed to afford a record of complement fixation in hog-cholera. It must not be understood that we claim to have obtained a specific antigen for the hog-cholera antibodies. It will require considerable work either to prove or disprove the specific nature of this antigen which we have developed. We have obtained an antigen which shows striking differences in its reaction toward normal hog, rabbit, and cow sera, and hyperimmune hog serum. This antigen is not present in the freshly prepared extract of mesenteric glands, but requires a definite period for development; it is not removed from such an extract by passage through an ordinary porcelain filter, but is removed by passage through the "F" bougie; finally it gradually disappears from the extract.

In the Wassermann reaction there is no analogy between the extract of syphilitic liver and true antigen, and yet the results of

this method of diagnosis have, on the whole (with certain reservations), been satisfactory.

We are continuing this work, especially in the direction of improving the method of preparing the antigen, and of increasing its sensitiveness.